Remarks

Claims 1, 3-17, 21, 30-34, 36, and 37 are pending in the subject application and currently before the Examiner for consideration. By this Amendment, Applicants have amended the specification to correct an inadvertent typographical error therein. Entry of the amendment is respectfully requested. Favorable consideration of the pending claims is respectfully requested.

In order to advance prosecution of the subject application, Applicant hereby requests that the Examiner contact Applicant's undersigned representative to arrange a telephonic interview with Applicant, Applicant's representative, the Examiner, and the Examiner's supervisor prior to the issuance of any further Office Action on the merits.

Applicant would like to bring to the Examiner's attention an Information Disclosure Statement listing references for consideration in the prosecution of the subject application which is being submitted in conjunction with the filing of this Amendment. Applicant notes that European Patent Application No. 0223618 and U.S. Patent No. 4,971,903 relate to nucleic acid sequencing procedures requiring the measurement of various components in the reaction effluent. European Patent No. 0425563 relates to amplification procedures. Applicant respectfully requests that the references be considered and made of record by the Examiner in the subject application.

Claims 1, 3-9, 15, 17, 21, and 30-34 are rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Foster (U.S. Patent No. 5,485,277). Claim 10 is rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Foster (U.S. Patent No. 5,485,277) further in view of Chang *et al.* (U.S. Patent No. 5,801,042). Claims 11-12 are rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Foster (U.S. Patent No. 5,485,277) further in view of O'Donnell (U.S. Patent No. 6,221,642). Claim 13 is rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Foster (U.S. Patent No. 5,485,277) further in view of Rosenthal *et al.* (WO 93/21340). Claim 14 is rejected under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Foster (U.S. Patent No. 5,485,277) further in view of Vind (U.S. Patent No. 6,159,687). Claim 16 is rejected

under 35 USC §103(a) as obvious over Tsien *et al.* (WO 91/06678) in view of Holzrichter *et al.* (U.S. Patent No. 5,620,854) further in view of Foster (U.S. Patent No. 5,485,277) further in view of Smith *et al.* (U.S. Patent No. 5,753,439). Applicant respectfully traverses each of these grounds of rejection.

Applicant respectfully maintains that the subject invention is not obvious over any combination of the cited references. The Examiner has again cited Tsien *et al* and Holzrichter *et al.* as primary references in each of the rejections of the claims. The teachings of the Tsien *et al.* and Holzrichter *et al.* references have now been supplemented with that of the Foster patent. All of the rejections in the outstanding Office Action rely on the combination of Tsien *et al.*, Holzrichter *et al.* and Foster; therefore, Applicant's comments regarding these references are directed to each of the rejections set forth in the Action.

The Examiner has maintained that it would be obvious to substitute the immobilized polymerase disclosed in the Holzrichter *et al.* patent into the sequencing method disclosed in the Tsien *et al.* reference. Applicant respectfully asserts that the Examiner's statements and position again overlooks the fact that the Tsien *et al.* method **will not work** if the polymerase is immobilized rather than the target polynucleotide, as the Tsien *et al.* method relies on the removal of unbound components with subsequent measurement of incorporated nucleotides being carried out by the detection of a fluorescent label. As the Examiner is aware, Tsien *et al.* disclose a DNA sequencing procedure carried out by measuring the stepwise incorporation of labeled nucleotides complementary to a target polynucleotide. There is an explicit requirement for the target polynucleotide to be immobilized (see in particular page 32, lines 9-36, and all of pages 33-34 of the Tsien *et al.* reference). There is also an explicit requirement for the nucleotides to comprise blocking groups, so that only one nucleotide can be incorporated at any one time. Detection of the incorporated nucleotide occurs only after the non-incorporated components, including the (unbound) polymerase, are removed during a washing step. Additional nucleotide incorporations can follow upon removal of the blocking group and upon the reintroduction of the various nucleotides and the polymerase back into the reaction chamber (see page 13, lines 23-29 of the Tsien *et al.* reference). As stated above, the Tsien *et al.* method requires that the nucleotides are blocked, to prevent further nucleotide incorporation and nascent strand synthesis. Blocking the nucleotides has the effect of stopping the

polymerase reaction (which is necessary in the Tsien *et al.* method to permit detection of the label), and in doing so, the polymerase disassociates from the target polynucleotide. The washing step in the Tsien *et al.* method (see, for example, page 12, lines 29-34 of the Tsien *et al.* reference) washes away all the non-incorporated nucleotides and the polymerase from the reaction chamber. Thus, if the skilled artisan were to modify the method of Tsien *et al.* so as to immobilize the polymerase (rather than the target polynucleotide), the result would be that, following incorporation of the blocked nucleotide, the target polynucleotide and any incorporated labeled nucleotide would dissociate from the polymerase and then would be removed from the reaction chamber during the washing step. It would then be impossible to detect the incorporation event because the nascent strand with the incorporated labeled nucleotide is washed away. The proposed modification would effectively render the method disclosed in the Tsien *et al.* reference **inoperable**. Moreover, the ordinarily skilled artisan would know that these problems would result from modifying the Tsien *et al.* method to immobilize the polymerase. Therefore, it would be inconceivable for the skilled artisan to modify, or even consider modifying, the teaching of Tsien *et al.* in the manner suggested by the Examiner. It is well accepted in patent law that if a proposed modification of the prior art in an effort to attain the claimed invention causes the art to become inoperable or destroys its intended function, then the requisite motivation to make the modification would not have existed. See *In re Fritch*, 23 USPQ2d 1780, 1783 n.12 (Fed. Cir. 1992) ("A proposed modification [is] **inappropriate** for an obviousness inquiry when the modification **render[s] the prior art reference inoperable** for its intended purpose." (emphasis added)). If the Examiner disagrees with Applicant's position concerning this issue as to the inoperability of the Tsien *et al.* method when modified to have the polymerase in the method immobilized, then Applicant respectfully asserts that the Examiner must clearly set forth his reasoning and explanation as to why he believes the proposed modification of the Tsien *et al.* method would result in an operable method.

The Examiner states that the Tsien *et al.* and Holzrichter *et al.* references do not teach a nascent polynucleotide being synthesized as a result of the polymerase reaction wherein the complimentary nucleotides are not labeled and the effect detected results from a conformational or mass change of the polymerase that occurs upon incorporation of the nucleotide. While the Examiner's statement is correct in that the cited references fail to teach the noted elements,

Applicant would like to clarify that Applicant's amended claim 1 does not refer to or recite detection resulting from a conformational or mass change of the polymerase. Rather, step (ii) of claim 1 makes it clear that what is detected is the interaction between these distinct elements: the polymerase enzyme, the target polynucleotide, and a nucleotide complementary to a nucleotide in the target polynucleotide. This clearly distinguishes the claimed invention from the method disclosed in the cited references, including the Tsien *et al.* publication, where the detection is carried out after the incorporation of the nucleotide onto the target polynucleotide (the polymerase enzyme no longer being present) and it is a label on the incorporated nucleotide that is detected.

The Foster patent is cited by the Examiner to supplement the teachings of the Tsien *et al.* and Holzrichter *et al.* references. Under the rejections, the Examiner states that the Foster patent teaches a polynucleotide being synthesized as a result of a polymerase reaction. However, Applicant respectfully asserts that the Foster patent does not teach synthesis of a polynucleotide via a polymerase reaction. Column 12, lines 30-47, of the Foster patent teaches the use of surface plasmon resonance (SPR) to detect the **hybridization** between different nucleic acid strands. There is no teaching or suggestion in the Foster patent of using SPR with a polymerase reaction. The Foster patent does not teach or suggest anything in regard to the use of SPR in nucleic acid sequencing or other reactions involving a nucleic acid polymerase. The disclosure in the Foster patent, as it relates to nucleic acids, is only directed to hybridization of one nucleic acid probe to another nucleic acid. Nucleic acid hybridization does not require or even involve the presence of a polymerase. Although there is one reference to "polymerase chain reaction" at line 34 of the Foster patent, this text is simply referring to a conserved gag gene which, as is stated in the Foster patent, is "commonly used in polymerase chain reaction detection procedures." However, that part of the disclosure in the Foster patent, at lines 30-47, still only pertains to nucleic acid probe hybridization. There is no teaching or suggestion in the Foster patent of detecting the incorporation of single nucleotides into a polymerase enzyme using SPR, or any other technique. Applicant again asserts that the hybridization of polynucleotides, as is disclosed in the Foster patent, has no relevance to nucleic acid sequencing or to a polymerase reaction in regard to the claimed invention and the use of SPR in the claimed method. Accordingly, Applicant respectfully asserts that the Foster patent does **not** overcome the deficiencies in the Tsien *et al.* and Holzrichter *et al.* references and, therefore, the

combination of references cited by the Examiner does not render Applicant's claimed invention obvious.

In regard to the rejection of claim 14 over Vind (U.S. Patent No. 6,159,687), Applicant notes that the Vind patent was filed March 18, 1998. This is after Applicant's foreign priority date of July 28, 1997. While Applicant realizes that the Vind patent claims priority to provisional applications filed April 25, and June 24, 1997, the Examiner has not established that the provisional applications contain the same disclosure which the Examiner cites the patent as teaching. Accordingly, Applicant respectfully asserts that if the Examiner is relying upon the filing dates of the provisional applications for purposes of citing the Vind patent as 35 USC §102(e)/103 prior art, then the Examiner should provide Applicant with a copy of the provisional applications and indicate where in the provisional applications there is support for the disclosure in the Vind patent being relied upon in the rejection. In the absence of such a showing, Applicant respectfully asserts that the rejection of claim 14 based on the Vind patent should be withdrawn.

In view of the above remarks, reconsideration and withdrawal of all of the rejections under 35 USC §103(a) is respectfully requested.

In view of the foregoing remarks, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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DRP/sl
Attachment: Information Disclosure Statement

In the Specification

Please substitute the following paragraph beginning on page 5, line 15, through to page 6, line 2:

An important aspect of the method of the present invention is the use of a polymerase enzyme immobilised onto a solid support. Immobilisation of the polymerase offers several important advantages for the success of this method. Firstly, the problem of random "noise" associated with measuring energy absorption in soluble molecules is reduced considerably. Secondly, the problem of noise from the interaction of any substrate (e.g. nucleotides) not directly involved with the polymerase is reduced, as the polymerase can be maintained within a specifically defined area relative to the field of measurement. This is particularly relevant if the technique used to measure the changes in radiation requires the measurement of fluorescence, as in TIRF, where background fluorescence increases as the nascent chain grows. Also, if SPR spectroscopy is used, the polymerase reactions are maintained within the evanescent wave field and so accurate measurements can be made irrespective of the size of [te] the polynucleotide. Finally, as neither the target polynucleotide nor the oligonucleotide primer is irreversibly attached to the solid surface, it is relatively simple to regenerate the surface, to allow further sequencing reactions to take place using the same immobilised polymerase.